REMARKS

I. Introduction

Applicants have reviewed and considered the Final Office Action dated December 28, 2005 and the new reference cited therewith. Applicants note that claims 1-2 are pending in the current application. Applicants respectfully request reconsideration of the above identified application in view of the remarks that follow. The amendments and remarks place the claims in an allowable form and, in the alternative, in better form for appeal.

II. Claim Rejections - 35 U.S.C. 112, Second Paragraph

The Examiner notes that the rejection of claim 1 under 35 U.S.C. 112, second paragraph, has been maintained due to Applicants' failure to modify the claim, presumably for the same reasons set forth in the July 1, 2005 Office Action. Contrary to the Examiner's assertion, claim 1 was modified in Applicants' September 14, 2005 response to delete the phrase "1:1 neutral complex" to state that the complex contains "one ion of the trace element for each molecule of the dicarboxylic alpha amino acid, with the molecule of the complex having a net zero charge." It is not understood how this language could be more clear. Applicants respectfully request withdrawal of this rejection.

III. Claim Rejections - 35 U.S.C. 112, Second Paragraph

The Examiner further notes that the rejection of claims 1 and 2 under 35 U.S.C. 112, first paragraph, has been maintained due to Applicants' failure to modify the claims, presumably for the same reasons described in the July 1, 2005 Office Action. Again, for the same extensive reasons outlined in Applicants' September 14, 2005 response, it is believed the phrase "an essential trace element" meets the written description. Such phrase is also described and defined on p. 8 of the specification.

In the interest of expediting prosecution, Applicants have now amended claim 1 to specify the essentially trace elements of zinc, copper, manganese, iron, cobalt, nickel, vanadium and molybdenum, as set forth on p. 8, lines 21-22 of the specification. No new matter has been added. Applicants therefore respectfully request withdrawal of this rejection.

IV. Claim Rejections - 35 U.S.C. 102(b)

A. Nikiforov et al.

Applicants acknowledge withdrawal of the rejection of claims 1-2 under 35 U.S.C. 102(b) as being anticipated by Nikiforov et al.

B. Godfrey

Applicants acknowledge withdrawal of the rejection of claims 1-2 under 35 U.S.C. 102(b) as being anticipated by Godfrey.

C. Kaczmarczyk et al.

Applicants acknowledge withdrawal of the rejection of claims 1-2 under 35 U.S.C. 102(b) as being anticipated by Kaczmarczyk et al.

D. Kirschner et al.

The Examiner has maintained the rejection of claims 1-2 under 35 U.S.C. 102(b). Applicants respectfully traverse this objection. It is the theory of the Examiner's rejection that Kirschner's ferrous glutamate complex inherently has the claimed complex with the specific ratio.

A rejection under 35 U.S.C. § 102(b) for anticipation, such as made by the Examiner in the instant case, necessarily implies that the invention sought to be patented has been, "patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of the application for patent in the United States,"

and therefore is not "new" - that there are no differences between what is claimed and what is disclosed in the prior art. Bearing this legal standard in mind, it is apparent that Kirschner et al. do not specifically name, describe or claim any particular, individual compound anticipating Applicants' claims, nor is there any suggestion by Kirschner that its iron compound is a neutral, 1:1 complex, and therefore capable of being used for any of Applicant's intended purposes, for example, meeting the dietary needs of humans and animals by providing a more bioavailable source of trace mineral.

The facts at hand are analogous to those presented in Application of Kalm, 378 F.2d 959 (CCPA 1967), a case of binding authority in this matter (a copy of which is enclosed for the Examiner's convenience). In Kalm, the claimed invention related to particular morpholine derivatives. Kalm, 378 F.2d at 960. Claim 3 was directed to the specific compound 2-cyclohexyl-3, 4-dimethylmorpholine. Id. According to the specification, Kalm's compounds were described as being useful as "selective central nervous system [CNS] depressants - being potent barbiturate potentiators." (Emphasis supplied). Id. According to Siemer, the compounds he disclosed had "a most marked anti-depressive action." Id. at 961.

The examiner rejected claims 1-3 under 35 U.S.C. § 102(e) as being anticipated by the Siemer patent. Kalm, 378 F.2d at 960-61. The CCPA (predecessor to the Federal Circuit) reversed the examiner and Board's rejection of the claims 1-3 under Section 102, stating that there appeared to be "no question that the Siemer patent does not specifically name, describe or claim any particular, individual compound anticipating appellant's claims, nor is there any suggestion by Siemer that any of his disclosed compounds is capable of depressing the central nervous system. Kalm, 378 F.2d 959, 962 (CCPA 1967). The Court noted that it was the Patent Office's position that Kalm's claimed compounds fell within the scope of the "genus" disclosed

by Siemer. Id. at 962-63. The Court disagreed. Instead, the Court determined that Siemer's genus was limited to compounds possessing properties "diametrically opposite" to the properties possessed by Kalm's genus of compounds. Id. at 963. The Court added:

> While it is not necessary that a reference disclose every property or attribute of a composition of matter to be a valid anticipation, appellant has found properties for his claimed compounds which are totally incompatible and inconsistent with, not merely complementary or in addition to, those attributed by Siemer to his compounds. It is our view that Siemer never intended to, nor does he, disclose compounds within the scope of appellant's claims.

Id.

In the present application, the Examiner argues that one of the numerous iron compounds disclosed in Kirschner has "the possibility" of disclosing each and every limitation of Applicant's claimed compounds. (12/23/05 Office Action, p. 6). However, as noted above, it is not sufficient for the Examiner to demonstrate an outside possibility that something may anticipate in order to satisfy the legal burden under Section 102(b). In fact, there is no indication in Kirschner whatsoever that its iron compound is a neutral, 1:1 complex, as required by Applicants' claims. Kirschner therefore does not anticipate, and Applicants' respectfully request that this ground of rejection be withdrawn.

E. Henry, Jr.

The Examiner has maintained the rejection of claim 1 under 35 U.S.C. 102(b). Applicants respectfully traverse this objection. It is the theory of the Examiner's rejection that there is a "possibility" that Henry's zinc aspartate could inherently be the same as Applicants' claimed compound. Again, as noted above with respect to Kirschner, the law provides that mere "possibilities" of anticipation are not sufficient for the Examiner to meet the requirements of Section 102(b). Here, there is no indication that the zinc aspartate compound of Henry is a

neutral, 1:1 complex, as required by the claimed invention. In fact, Henry teaches away from the claimed invention in noting that the zinc compounds which can be used in his invention, "can be any of the commonly used forms." (Col. 7, lines 13-14). Henry, Jr. therefore does not anticipate the claimed invention. Accordingly, Applicants respectfully request that this ground of rejection be withdrawn.

F. Weitzel et al.

The Examiner has issued a new rejection of claims 1-2 under 35 U.S.C. 102(b) as being anticipated clearly by Weitzel et al.[1] Applicants herewith attach a complete copy of this article, along with an English translation of the same.

The Weitzel article describes the results of a study on the effect of various zinc compounds on blood sugar. In this regard, Weitzel describes the synthesis of zinc gluconate, "recovered from gluconolactone and $ZnCO_3$, accumulated as a grainy, crystalline precipitate and corresponded to the composition cited in the literature: $Zn(gluconate)_2 + 5H_2O$." (Weitzel, p. 3). Thus, Weitzel actually teaches 1:2 complexes of zinc and gluconic acid. Since the claimed invention is directed to neutral, 1:1 neutral complexes, Weitzel does not anticipate, and this ground of rejection should be withdrawn.

V. Conclusion

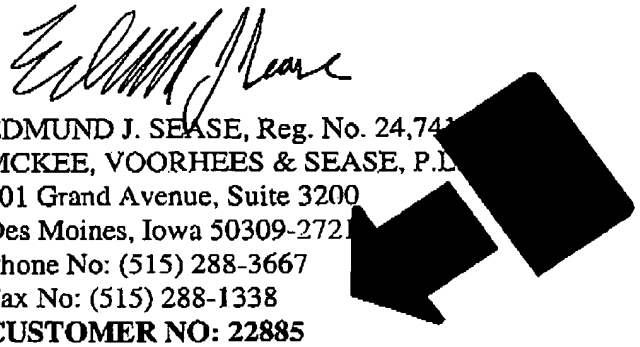
For all of the above-stated reasons, it is believed the present application is in a condition for allowability. Allowance is respectfully requested.

[1] Although the current application has been pending for over two years and has already been up on appeal, and this is the first instance the Weitzel reference has ever been cited, the Examiner has made the rejection final, claiming "Applicant's amendment necessitated the new ground(s) of rejection."

No fees or extensions of time are believed to be due in connection with this amendment; however, consider this a request for any extension inadvertently omitted, and charge any additional fees to Deposit Account No. 26-0084.

Reconsideration and allowance is respectfully requested.

Respectfully submitted,



EDMUND J. SEASE, Reg. No. 24,74[illegible]
MCKEE, VOORHEES & SEASE, P.L[illegible]
801 Grand Avenue, Suite 3200
Des Moines, Iowa 50309-272[illegible]
Phone No: (515) 288-3667
Fax No: (515) 288-1338
CUSTOMER NO: 22885

Attorneys of Record

- JPB/WKM -

Blood Sugar Effect of Zinc Compounds

Günther Weitzel, Franz-Josef Strecker and Ursula Roester

Medical Research Institute (Department of Biochemistry)
of the Max-Planck-Society, Goettingen

(Received by the editorial staff on March 4, 1953)

Only a few studies are available on the effect of zinc compounds on the carbohydrate metabolism, apart from zinc-containing insulin preparations. In 1892 Italian authors[1] wrote about significant glycosuria in dogs that received 0.5-1 g of zinc daily in their food. In 1918, Salant and Wise[2] reported that feeding or injection of zinc salts caused hyperglycemia and glycosuria in rabbits, dogs and cats. With orally administration of zinc acetate, rabbits exhibited glycosuria and albuminuria at 335 mg zinc/kg, but not yet at 30-100 mg Zn/kg. Zinc malate administered subcutaneously caused glycosuria and albuminuria at doses of 50-100 mg zinc/kg; intravenously, doses of 9-10 mg Zn/kg in the form of zinc malate could just barely produce slight glycosuria, with blood sugar values usually hovering around 200 mg%. Almost all animals had albuminuria and died over a period of 2-9 days after receiving the zinc. Cats reacted with glycosuria after subcutaneous injection of 25-100 mg Zn/kg in the form of zinc malate; dogs received 15-26 mg Zn/kg in the form of zinc malate intramuscularly, resulting most often in glycosuria. But these dogs survived the zinc injection for only 1-5 days.

The effect of lower doses of injected zinc salts on the blood sugar of dogs was studied by Sanfilippo[3] (0.87 mg Zn/kg each IM and IV in the form of zinc chloride, -bromide, -iodide, -nitrate, -sulfate, -lactate and -acetate). He found no changes in the normal glucose level. Berenshtein and Shkolnik[4] also observed no changes in the blood sugar of rabbits or dogs after subcutaneous injection of zinc sulfate or zinc acetate in doses of 100-200 (Zn/kg, but did after higher doses (0.5-5.0 mg Zn/kg).

In summary, these studies show that it takes high doses of zinc to cause hyperglycemia and glycosuria. These are usually very toxic however, so that the animals often perish while still in the acute test. When administering lower doses, glycosuria disappears while hyperglycemia

[1] L. D'Amore, C. Falcone and L. Maramaldi, C.R. Séances Soc. biol. Filiales Associées 4, 335 [1892].
[2] W. Salant and L. Wise, J. biol. Chemistry 34, 447 [1918].
[3] G. Sanfilippo, Arch. Farmacol. sperim. 73, 87 [1942].
[4] F.Y. Berenshtein and M.J. Shkolnik, Fizio.Z. 37, 120 [1950]; zit..n.: Excerpta Med. 6, Sect. III, No. 10, 447 [1952]; Chem Abstr. 45, 10330 [1951].

is initially still present, but also fails to materialize when doses are lowered further. According to the literature, with parenteral administration, that dose which still produces marked hyperglycemia, probably lies in the range of 1-5 mg Zn/kg. It is not clear from the mentioned studies, however, whether the extent of the zinc effect is varied by the chemical structure of the administered zinc compound or whether its composition is of no importance in the ..... of hyperglycemia.

We studied the effect of different zinc-bound acid residues for level and duration of zinc-hyperglycemia; in particular, we checked to see whether suitable organic residues, e.g. complex zinc compounds, can achieve an increase in blood sugar with lower doses of zinc, i.e. whether doses of 1 mg Zn/kg down to fractions of a (Zn/kg are still capable of affecting the blood sugar.

This paper deals with

1. zinc salts of nitrogen-free acids, administered to rabbits parenterally in doses of 1 mg down to 0.001 (Zn/kg. The following zinc salts were tested:

chloride	pyrophosphate	malate	gluconate
sulfate	citrate	maleate	glucuronate
acetate	tartrate	pyruvate	ascorbate

2. zinc salts of ∀-amino acids in which the strength of the coordinative zinc binding can largely be modified depending on type and number of the amino acid residues. There is no information in the literature on the effect on blood sugar of zinc-amino acid complexes. We tested orally, intramuscular and intravenous administration of zinc glycine, zinc alanine and zinc glutamate using doses of several mg zinc/kg to as low as 0.001 (Zn/kg.

Method and substances

All tests performed on rabbits that had fasted for 24 hrs. Dissolution in 0.9% NaCl-solution of the zinc compounds to be injected, adjustment of the pH to 6.4-7.0. Using only freshly-prepared solutions. Blood sugar determinations according to Hagedorn-Jensen or Fujita and Iwatake[5]. Number and time intervals between blood sampling are shown in the figures.

The zinc compounds to be tested were produced in solid form with the exception of zinc chloride, zinc sulfate and zinc acetate (commercial p.a.-preparations) and zinc pyrophosphate (see below). The zinc content was determined by direct microtitration with ethylenediaminetetraacetic acid as disodium salt (complexon III) according to Flaschka[6]. Indicator: Eriochrome Black T. Calculation of the substance quantity required for the animal test based on the zinc analysis*.

[5] A. Fujita and D. Iwatake, Biochem. Z. 242, 43 [1931].

[6] H. Flaschka, Mikrochemie 39, 38 [1952].

* We thank Dr. A.-M. Fretzdorff (Medical Research Institute, Department of Biochemistry) for carrying out the zinc analyses.

The zinc salts were recovered via reaction of the acids in question – which were dissolved in water – with the calculated amount of zinc carbonate, if necessary with precipitation of the formed zinc salts using ethanol. Rather than using the very slightly soluble $Zn_2P_2O_7$ which is unsuitable for injections, we used acidic zinc pyrophosphate, which was obtained in dissolved form from $ZnCO_3$ and aqueous pyrophosphoric acid. Zinc citrate and zinc malate were prepared in the same manner and were precipitated out of the aqueous solution by adding an equal volume of ethanol. Zinc tartrate, zinc maleate and zinc pyruvate precipitated out of water spontaneously as amorphous precipitates. Due to their slight solubility, these three salts could only be applied in doses of a maximum of 100 γ or 10 γ Zn/kg (see below). Zinc gluconate, recovered from gluconolactone and $ZnCO_3$, accumulated as a grainy, crystalline precipitate and corresponded to the composition cited in the literature[7]: $Zn(gluconate)_2 + 5\ H_2O$. Upon recovery of zinc glucuronate from gluconolactone and $ZnCO_3$ we observed the following fact: In order to avoid recovering a zinc salt which immediately decomposes via hydrolysis at neutral pH, we prepared with excess glucuronic acid (Zn : glucuronic acid = 1 : 3.5), whereby we obtained the salt in yellow crystals from 50 percent ethanol. Hoffmann-La Roche (Basel) kindly provided us with zinc ascorbate in the form of a yellow amorphous powder with a zinc content of 18.3%.

Depending on the manufacturing process, glycine, alanine and glutamic acid yield zinc salts of varying composition. These salts dissolve clearly in water in a neutral reaction or are also more or less rapidly separated out by water and form flocculent precipitates. Zinc-amino acid complexes, which become turbid and release zinc hydroxide very soon after they dissolve in aqueous solution at neutral pH, are not suitable for injection purposes. In our experiments, we only used those zinc-amino acid complexes whose stability relative to water was sufficiently great so as to exclude any decomposition at neutral or slightly alkaline pH. However, there are large variations of activity among such complexes; only a few zinc complexes are suitable for bringing about sizeable increases in blood sugar at doses below 100 γ Zn/kg. Unless otherwise noted, the experimental results with zinc-amino acids described below refer to complexes with high activity.

Results

1. Zinc salts of N-free acids

Zinc chloride and zinc sulfate

Intravenous injection of $ZnCl_2$ in doses of 1 mg, 100 (, 10 (, 1 (and 0.01 (zinc/kg did not lead to significant increases in blood sugar. Only once, after 1 mg zinc/kg, blood sugar increased from 112 to 132 mg% after injection. Two other animals did not show this effect after the same dose. $ZnSO_4$ presented similar results: 1 mg zinc/kg triggered marked hyperglycemia in one animal directly after the injection, with a peak of 164 mg% after 25 min., another animal reacted with normal values to the same dose. Lower zinc doses, down to 0.01 (zinc/kg (as with $ZnCl_2$), were lacking clear and reproducible blood-sugar increasing effects.

Concurring with information in the literature,[2,3,4] $ZnCl_2$ and $ZnSO_4$ with 1 mg zinc/kg apparently represent the lower limit of a hyperglycemia-evoking dose. Doses below 1 mg zinc/kg

[7] Criesbammer, Arch. Pharmaz. 215, 204 [1879]

no longer show any noticeable effect, but on occasion a slight increase in blood sugar can still be observed even with very small doses (e.g. 1 (Zn/kg). What is remarkable is that the animals tolerate the intravenous injection of these strongly-ionizing zinc salts without acute manifestations; we could not even detect any late effects.

Zinc acetate

Compared to $ZnCl_2$ and $ZnSO_4$, the blood sugar curves after zinc acetate IV change insofar as slight blood sugar increases (usually 20-30 mg%) occur somewhat more often, but not reliably reproducibly, that are not dose-dependent however. Because after just a few (and even after 0.001 (zinc/kg in the form of zinc acetate IV, blood sugar elevations can be observed. See the example in Fig. 1 containing curves for 0.01 (and 0.001 (Zn/kg IV.

[graphic]	Fig. 1. 0.01 (Zn as zinc acetate IV ———— K 288; 0.001 (Zn as zinc acetate IV ---------- K 298.

Zinc pyrophosphate

In the studies with acidic zinc pyrophosphate in doses of 1 mg Zn/kg to 0.01 (Zn/kg we did not see a single case (we studied 17 animals) of blood sugar increase exceeding the normal fluctuations.

Zinc citrate

Compared to $ZnCl_2$, $ZnSO_4$ and zinc acetate, there is no increase in the blood-sugar elevating effect when using zinc acetate. Based on 14 rabbit tests, we rather have the impression that zinc citrate affects the blood sugar only in exceptional cases, and then clearly not in a dose-dependent manner, since it is specifically the doses of 1 mg and 100 (zinc/kg that yielded downright empty curves.

Zinc tartrate

Zinc tartrate which, because of its solubility, could only be tested in doses of 10 (, 1 (and 0.01 (zinc/kg, behaved the same way as zinc citrate.

Zinc malate

The intravenous injection of zinc malate (18 rabbit tests) led to blood sugar elevations in about half of the cases with all tested doses. As an example, Fig. 2 shows the blood sugar curves we obtained after injecting 1 mg and 1 (zinc/kg as zinc malate.

[graphic]	Fig. 2. 1 mg zinc/kg as zinc malate ———— K 249; 1 (zinc/kg as zinc malate ---------- K 248.

Zinc maleate

Because this salt is difficult to dissolve, we did not test zinc maleate in a dose of 1 mg Zn/kg in order to avoid having to inject too big of a volume. Doses between 100 (and 0.01 (Zn/kg initially caused blood sugar elevations in the majority of cases, but they were so small that we prefer not to attribute them with certainty to the injected zinc complexes.

Zinc pyruvate

Just as zinc maleate, zinc pyruvate difficult to dissolve, and we could not arrive at a volume suitable for injecting a dose of 1 mg Zn/kg. Intravenous injection of zinc pyruvate in doses of 100 (, 10 (and 1 (Zn/kg did not elicit a usable effect on blood sugar in 12 rabbits.

Zinc gluconate

After injecting zinc gluconate (14 rabbits), significant increases in blood sugar occurred only rarely. What is remarkable is that it is precisely the lowest doses (0.01 (Zn/kg) that produced the most pronounced blood sugar elevations; see Fig. 3.

[graphic]	Fig. 3. 0.01 (Zn/kg IV each as zinc gluconate. K 278 ———— and K 315 ----------

Zinc glucuronate

Zinc glucuronate (14 rabbit tests) behaves approximately the same way as zinc gluconate. Fig. 4 shows, as examples, two curves illustrating the blood sugar course after injection of 1 (Zn/kg each as zinc glucuronate.

[graphic]	Fig. 4. 1 (Zn/kg IV each as zinc glucuronate. K 242 ———— and K 272 ----------

Zinc ascorbate

Twenty-four rabbit tests with zinc ascorbate yielded the same results as tests with zinc gluconate and zinc glucuronate. Some of the blood sugar elevations were questionable, others unambiguous, but they were dose-independent. Examples in Fig. 5 show the blood sugar curve after injection of 100 (Zn/kg and 0.01 (Zn/kg as zinc ascorbate.

[graphic]	Fig. 5. 100 (Zn/kg IV each as zinc ascorbate, K 324 ———— 0.01 (Zn/kg IV as zinc ascorbate, K 241 ----------

Control tests

In the case of those above-mentioned zinc complexes that proved to have an effect on blood sugar, we performed control tests with the respective free acids or their salts. Sodium malate,

sodium glucuronate and sodium ascorbate as well as free ascorbic acid did not elevate blood sugar with the doses in question. Only in the case of sodium gluconate, slight initial blood sugar elevations occurred in the range from 8 mg down to 8(gluconic acid/kg which, however, were not present with equivalent doses of calcium gluconate.

2. Zinc salts of ∀-amino acids

The test results are compiled below in three groups according to the administered level of zinc per kg body weight:

A. More than 1 mg Zn/kg. B. 1 mg up to 1 (Zn/kg. C. Less than 1 (Zn/kg.

A. Doses above 1 mg Zn/kg

[graphic]	Fig. 6. 14 mg Zn/kg orally as zinc glycine, K 60 ———— 10 mg Zn/kg orally as zinc glutamate, K 66 ----------

Fig. 6 shows the blood sugar curve after orally administration of zinc glycine and zinc glutamate (14 and 10 mg Zn/g, respectively): During the first 10 hours after intake of the zinc compound, considerable hyperglycemia occurs, accompanied by glucosuria and albuminuria. During the following days as well, hyperglycemic conditions, interrupted by normal or even subnormal glycemic values, were repeated observed. Slight glucosuria was still detectable on the fourth day after the zinc feeding. Further studies with zinc glycine and zinc glutamate revealed the same picture as the curves represented in Fig. 1 whose course is typical of the dysregulation of blood sugar in the case of zinc poisoning; the doses used are highly toxic and almost all treated animals perished after 2-10 days.

Salant and Wise[2] needed 335 mg Zn/kg orally in the form of zinc acetate to produce glucosuria in rabbits, which was not achieved with 30-100 mg Zn/kg. Using zinc-amino acid complexes, it is possible, as shown in Fig. 6, to bring about glucosuria with considerably lower doses of zinc. This is probably, to a large part, attributable to the fact that, contrary to zinc acetate, the employed zinc-amino acid complexes are very well resorbed. For instance, hardly

any zinc ions are released in the gastrointestinal tract from stable zinc-glycine complexes, the typical heavy-metal effects are missing. Zinc glycine, which dissolves clearly at neutral pH, for instance, causes neither a metallic taste nor nausea or vomiting in humans after orally ingestion.

[graphic]

Fig. 7. 2.5 mg Zn/kg IM each as zinc alanine, K 207 ——— and K 295 ----------

In Fig. 7, two blood sugar curves are shown after intramuscular administration of 2.5 mg Zn/kg each as zinc alanine. The beginning blood sugar increase is already detectable 15 minutes after the injection; hyperglycemia lasted for 4-5 hours. The course of the blood sugar curve for the one animal (K 207) again showed slight hyperglycemic values 6-9 hours after the injection. These animals survived.

Intramuscular administration of amino acid-zinc complexes in higher doses, e.g. 28 mg Zn/kg as zinc glycine or 20 mg Zn/kg as zinc glutamate, had the same effect on blood sugar and general condition of the animals as described in Fig.6 for orally administration. Of 6 animals, 5 perished 3-5 days later.

[graphic]

Fig. 8. 6 mg Zn/kg IV as zinc glutamate, K 65 ———
8.5 mg Zn/kg IV as zinc glycine, K 62 ----------

Fig. 8 shows the course of blood sugar curves in the case of intravenous injection of several mg Zn as zinc glycine (8.5 mg Zn/kg) and zinc glutamate (6 mg Zn/kg). We observed hyperglycemic values in both cases, as well as glucosuria and albuminuria, as long as 80 hrs. after injection, interspersed with decreases in blood sugar down to approx. 50 mg%. Intravenous administration of zinc in quantities above 5 mg Zn/kg as inner zinc complexes of ∀-amino acids led to the death of 7 out of 8 animals. K 62 in Fig. 8 was lost after 80 hrs.; in parallel tests using the same dose, the animals died 60 hrs. and 100 hrs. after the injection. K 65 (see Fig. 8; 6 mg Zn/kg) was the only one to withstand the zinc load and survived.

With intravenous administration of 5 to 1 mg Zn/kg, extent and duration of hyperglycemia decreased, and toxicity abated significantly. Four out of 5 animals survived.

B. Doses of 1 mg to 1 (zinc/kg

[graphic]	Fig. 9. 1 mg Zn/kg orally as zinc glycine, K 293 ———— 50 (Zn/kg IM as zinc glycine, K 288 ----------

As shown in Fig. 9, even a dose of 50 (Zn/kg as zinc glycine, administered intramuscularly, caused hyperglycemia lasting several hours. Orally, 1 mg Zn/kg as zinc glycine also had a pronounced effect. The lowest still-effective dose administered orally was not tested due to the unclear resorption conditions of the rabbit intestines.

[graphic]

Fig. 10. 850 (Zn/kg IV as zinc glycine, K 76 ————
141 (Zn/kg IV as zinc glycine, K 90 ----------

[graphic]	Fig. 11. 14.1 (Zn/kg IV as zinc glycine, K 78 ——— 3.5 (Zn/kg IV as zinc glycine, K 96 ----------

Figures 10 and 11 show blood sugar curves after intravenous injection of zinc glycine in the range from 850 (to 3.5 (Zn/kg. These doses, too, still produce marked hyperglycemia that is detectable as early as 15 min. after injection and can reach values between 160 and 200 mg% within the first hour. In general, the blood sugar elevations still persist for several hours after the injection.

C. Doses smaller than 1 (zinc/kg

[graphic]

Fig. 12. 0.5 (zinc/kg IM as zinc glycine, K 246 ----------
0.5 (zinc/kg IV as zinc glycine, K 296 ———

[graphic]

Fig. 13. 0.01 (Zn/kg IV as zinc glycine, K 276 ———
0.001 (Zn/kg IV as zinc glycine, K 203 ----------

Figures 12 and 13 show tests with parenteral administration of less than 1 (Zn/kg. By progressively lowering the zinc dose, the level and duration of blood sugar elevation drop, hyperglycemia manifests itself as a steep blood sugar increase of short duration, that almost always occurs immediately after injection and is often followed by a second, weaker fluctuation.

Initial hyperglycemia after these remarkably low zinc doses occurs more or less regularly, depending on the type of administered zinc-glycine complexes, and almost without exception in the case of the best-suited zinc-glycine complexes (70 rabbit tests with doses below 1 (Zn/kg as zinc glycine). The lowest limit of effectiveness was not tested, but a random test with 0.0001 (Zn/kg of the most effective zinc-glycine complex still caused a marked increase in blood sugar.

Discussion of the Results

1. Zinc salts of N-free acids

Compared to the literature[1,2,4] dealing with hyperglycemia and glucosuria after administration of high and toxic quantities of zinc, the present studies show that zinc salts of nitrogen-free acids in doses of 1 mg zinc/kg IV and below can trigger slight increases in blood sugar that do not occur regularly, however. It is remarkable here that the smallest doses, e.g. 1 (or 0.01 (Zn/kg, can still trigger an initial increase in blood sugar, and that in the range from 1 mg to 0.01 (Zn/kg IV there is no dose dependence. Based on control tests with free acids or their sodium salts, the blood-sugar increasing effect has to be attributed to the zinc.

The differences in effectiveness between inorganic and organic zinc salts, or between highly-complex, slightly-complex and non-complex zinc salts, are not pronounced enough to draw conclusions to possible connections between the type of metal-binding and the blood sugar-increasing effect. Nevertheless, the curves give the impression that the structure of the acid residue bound to the metal is not unimportant. The fact that very low zinc doses, e.g. 1 (or 0.01 (Zn/kg, can affect the blood sugar level seems conspicuous and biologically remarkable since they are quantities that lie within the range of physiological orders or magnitude and — in our studies — are almost always bound to acid residues commonly found in cells.

The zinc doses administered by us, which did not exceed 1 mg Zn/kg, did not exhibit any

toxicity. In dogs, however, Vallee et al.[8] — after intravenous injection of 4 mg/kg of zinc gluconate — observed paralysis of the hind legs, reduced tendon reflexes and general limpness, while 2 mg/kg of zinc gluconate was well tolerated in dogs and humans.

2. Zinc salts of ∀-amino acids

An overview of the results achieved with zinc-amino acid complexes presents a significantly different picture compared to the only slightly-pronounced blood-sugar elevating properties of the zinc salts of nitrogen-free acids.

Looking first at those of our studies using zinc-amino acid complexes, in which more than 1 mg zinc/kg was administered, we find significant hyperglycemia in all cases, sometimes even glucosuria. Compared to the zinc doses used by Salant and Wise[2] — 335 mg Zn/kg as acetate orally, 25-100 mg Zn/kg as malate subcutaneously, 15-26 mg Zn/kg as malate intramuscularly and 9-10 mg Zn/kg as malate intravenously — the quantities of zinc used in our studies are significantly lower. The lowest zinc dose limit of the American authors roughly corresponds to the upper limit of the zinc doses studied by us. The effect of the zinc-amino acid complexes, which is significantly higher than that of the zinc salts of nitrogen-free acids, can most likely be explained in part by the good resorbability of these compounds in the case of oral administration, as already mentioned.

The masking of the heavy metals by firm coordinative bonding, as is the case with the zinc-amino acid complexes used, seems to contribute to the increase in the zinc effect even in the case of parenteral administration. We were not able to observe a variation in the zinc effect attributable to the type of the amino acid residue with doses above 1 mg Zn/kg as far as solid complexes were concerned that remained clearly dissolved at neutral pH. In terms of their hyperglycemic effectiveness, there was practically no difference between zinc glycine, zinc alanine and zinc glutamate in mg-doses. Glucosuria was only observed if hyperglycemia was present at the same time. A reduction of the renal threshold for glucose could not be demonstrated.

While zinc-amino acid complexes in doses between 1 and 10 mg Zn/kg often cause a dysregulation of the blood sugar lasting for several days, the test results involving zinc doses below 1 mg Zn/kg, all the way down to fractions of a (Zn/kg, change in three different directions:

1. Level and duration of hyperglycemia decrease with the lowering of the zinc dose, but an increase in blood sugar continues to occur right after injection. At doses below 1 (Zn/kg, dose dependence can no longer be detected.

[8] B.L. Vallee, R.G. Fluhart and J.G. Gibson, IV. Internat. Cancer Research Congress, zit. n. Physiol. Rev. 29, 375/376 [1940]

2. Toxicity decreases proportionately to the continuing lowering of the dose.

3. Subtleties in the chemical structure of the complex together with the specificity of the amino acid residue become apparent.

Point 3. deserves particular attention since here clues can be found to the relationships between the special structure of the complex and the hyperglycemizing effect. The most important prerequisite for triggering an increase in blood sugar with zinc-amino acid complexes in (-doses is, as mentioned earlier, that complexes be involved that do not release any zinc hydroxide at neutral pH in aqueous solution, even after sitting for several days. On the other hand, testing of some zinc-amino acid complexes with particularly strong metal binding such as zinc histidyl-histidine, zinc cysteine and zinc glutathione showed that these complexes do not affect blood sugar in doses below 1 mg Zn/kg. But glycine can also be used to produce stable zinc complexes that do not increase blood sugar in doses of several 100 (Zn/kg.

When comparing zinc-amino acid complexes with the zinc salts of N-free acids (see above) with regard to the hyperglycemizing effect, the following picture emerges: While the blood sugar increases after administration of zinc salts of N-free acids in doses of 1 mg Zn/kg and below are very slight, the same doses of suitable zinc-amino acid complexes produce significantly stronger and regularly-occurring effects. Furthermore, in the range from 10 mg Zn/kg down to approx. 1 (Zn/kg, there is a relationship between level of the dose and extent of the effect. With doses between 1 (and 0.001 (Zn/kg, a gradation of the effect as a function of the administered amount of zinc is no longer discernable; apparently, the effect is then influenced to a larger degree by individual differences in the metabolic conditions of the test animals.

Control tests with amino acids: In the literature[9,10,11] there are observations on the hyperglycemizing effects of amino acids where, however, incomparably higher doses are required to produce increases in blood sugar than is the case in our studies. The control tests we conducted with amino acids (testing of all amino acids that were also administered as zinc salts in corresponding dosages) showed that the blood sugar increases achieved with zinc-amino acid complexes are attributable to the complexly-bound zinc. Proof for this is the above-mentioned

[9] L. Pollack, Biochem. Z. 127, 120 [1922]
[10] M. Chikano, Biochm. Z. 205, 154 [1929]
[11] E.G. Schenk, Naunyn-Schmiedebergs Arch. exp. Pathol. Pharmakol. 167, 201 [1932]

fact that we have zinc complexes of various composition from the same amino acid, of which some are effective at doses of less than 1 mg Zn/kg, while others do not affect the blood sugar.

Toxicity: While the doses (no higher than 1 mg Zn/kg) administered during testing of the zinc salts of N-free acids revealed no injury to the test animals (see above), zinc salts of amino acids administered in doses higher than 1 mg Zn/kg produced pronounced toxic symptoms which were not completely eliminated even at doses of 1 mg Zn/kg and below. Here is a short summary of our observations on the toxicity of these compounds:

The general condition of the test animals after injection of zinc-amino acid complexes is dependent on the level of the zinc dose. Administration of zinc in quantities above 1 mg Zn/kg as zinc glycine or zinc glutamate led to a collapse-like state that became more severe the higher the dose and sometimes lasted for several days, and during which it was extremely difficult to draw blood from the cold ears. The animals sat in a hunched position, their breathing was accelerated. Approximately two thirds of the rabbits did not recover from this state and perished after 48-120 hours after most of them suffered pareses of the hind legs and sometimes also cystoplegia. Doses of less than a few mg Zn/kg resulted in death as late as 8-14 days later, with the animals in severely reduced nutritional condition. Of the remaining rabbits, only a small number tolerated the cited zinc doses without harm to their general condition, the rest recovered slowly and survived without apparent late symptoms. In the case of high zinc doses, the manner of application — IV, IM or stomach tube — was irrelevant as far as the extent of the toxic manifestations was concerned.

Zinc doses of 1 mg to approx. 20 (/kg in the form of the above-mentioned amino-acid complexes caused significantly fewer cardiovascular impairments which decreased in proportion to the administered dose. When the dose was decreased, the number of animals displaying no toxic manifestations increased. Few animals died in reduced condition after 10-14 days.

Zinc doses below 20 (/kg led to toxic general changes in exceptional cases only. Such as exception may be the occurrence of a complete flaccid paralysis of the hind legs with cystoplegia a5 min. after injection of 0.35 (Zn/kg IV as zinc glycine (K 122).

For comparable doses of 1 mg Zn/kg and below, it can be said that zinc salts of amino acids are generally more toxic than zinc salts of nitrogen-free acids.

No reliable statements can be made, even today, about the mode of action that brings about blood sugar increase caused by zinc compounds. Clues to a possibly underlying process might be

found in older studies by Häusler and Schnetz[12]: These authors studied, on isolated frog livers, the effect of metals on the normal glycogenolysis and adrenalin-raised glycogenolysis. They found that zinc (in addition to Cu and Hg) in certain concentration ranges (10^{-4} to 10^{-8} millimol metal salt/L in the perfusion fluid) the sugar release from the frog liver increases markedly, but not in the case of other, higher (10^{-2} to 10^{-3} millimol $ZnSO_4$/L) or lower (10^{-7} millimol $ZnSO_4$/L) concentration ranges. However, if adrenalin was added to the perfusion fluid along with metal, zinc completely inhibited the adrenalin-generated rise of the glycogenolysis in the blank test. From these studies by Häusler and Schnetz[12] it follows that zinc, in very small concentrations, can have a glycogenolytic effect at least on the isolated liver of a cold-blooded animal, with this effect occurring without the involvement of adrenalin.

In further studies, Schnetz[13] came to the conclusion that zinc, cadmium and copper salts on the whole animal clearly diminish the adrenalin-hyperglycemia, and that the normal blood sugar level "is not significantly affected by the metals mentioned." This is contrary to the findings of Berenshtein and Shkolnik[4], who observed an increase in adrenalin-hyperglycemia when $ZnSO_4$ is injected at the same time.

This leads us to think, in interpreting the blood sugar increases observed by us after administration of very low doses of zinc, of an effect of corresponding ferment systems that contain zinc in the molecule or are activated by zinc. Phosphatase activations, for instance, may be considered here, since generally phosphate transfer by bivalent metals is increased and zinc is said to be included in the effective group of phosphates[14,15,16].

In view of the sparse and inconsistent information in the literature, we wanted to create a reliable experimental basis regarding the question of the hyperglycemizing effect of zinc compounds and, additionally, examine the heretofore unknown blood-sugar increasing effects of very low zinc doses. The 13 figures in this paper are the result of about 500 rabbit tests and each one is characteristic of a larger test series.

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[12] H. Häusler and H. Schnetz, Biochem. Z. **275**, 204 [1935]
[13] H. Schnetz, Naunyn-Schmiedebergs Arch. exp. Pathol. Pharmakol. **178**, 420 [1935]; Klin. Wschr. **15**, 646 [1936]
[14] R. Cloetens, Biochem. Z. **308**, 37 [1948]
[15] L. Massart and L. Vandendriessche, Naturwiss. **28**, 143 [1940]; R. Dufait and L. Massart, Naturwiss. **29**, 651 [1941]
[16] V. Sadasivan, Arch. Biochemistry **28**, 100 [1950]; Nature [London] **170**, 421 [1952]

As shown above, zinc salts of 12 N-free acids in doses of 1 mg Zn/kg and below are not capable of achieving strongly pronounced effects on the blood sugar level. In view of our numerous tests, we would like to consider this opinion as final. Zinc-amino acid complexes, on the other hand, deserve further intensive investigation, especially the chemical aspect, since until now their effectiveness in (-doses was not known any more than the fact that zinc-amino acid complexes of the same amino acid but having a different composition, can behave in biologically different ways. The latter circumstance is not very noticeable yet at high doses (above 1 mg Zn/kg), but at lower doses (below 1 mg Zn/kg) significant differences between the individual complexes can be observed. In another paper, we will report on the results we obtained in the study of the relationships between hyperglycemizing effect and structure of coordinative zinc binding.

Further pursuit of these relationships should be of particular interest for understanding the biological behavior of zinc-containing natural substances such as hormones and ferments, because in the zinc-amino acid complexes we have simple model substances in which the possible variations in zinc formation are relatively well-assessable and easy to access preparatively. Based on our test results, we now have to consider that in the case of zinc — just as with other metals — subtleties in the complex-structure can greatly affect the behavior of zinc compounds in the metabolism. Therefore, the findings reported in this paper regarding the blood-sugar elevating effect of (-doses of suitable zinc-amino acid complexes lead to the question of whether nature also makes use of this blood-sugar elevating principle. This seems to be the case because we[17] have been able to show in the meantime that hyperglycemizing extracts from pancreas and stomach mucosa regularly contain zinc bound in a complex that is apparently involved in the blood-sugar elevating effect in these extracts.

We thank Mrs. Gertrud Langenberg and Miss Käthe Büttner for their understanding and tireless cooperation.

Summary

The following zinc salts were administered to rabbits intravenously in doses of 1 mg zinc/kg down to 0.001 (zinc/kg and their effect on blood sugar studied: Zinc chloride, sulfate, acetate, pyrophosphate, citrate, tartrate, malate, maleate, pyruvate, gluconate, glucuronate and ascorbate. We tested, in the same manner, the complex zinc salts of glycine, alanine, glutamic acid and some

[17] G. Weitzel et al., Naturw., 40 [1953], in print.

other ∀-amino acid in doses of several mg zinc/kg down to 0.0001 (Zn/kg (administered not only intravenously, but also orally and intramuscularly).

Most of the tested zinc salts of N-free acids can cause slight initial blood sugar increases in the above-mentioned dosage range, but they do not occur regularly and their extent is not dose-dependent. Even very small doses such as, for example, 1 (and 0.01 (Zn/kg can cause an increase in blood sugar.

Zinc-amino acid complexes in doses above 1 mg Zn/kg cause considerable hyperglycemia, possibly even glucosuria, and are very toxic in most cases. With doses below 1 mg Zn/kg, differences in the structure of the complexes become apparent: Effective and ineffective zinc complexes can be produced from one and the same amino acid, differing in their composition.

Even fractions of one (Zn/kg of highly effective zinc complexes can result in marked blood sugar increases shortly after injection.